

Multivariate morphometrics of Vesper mice (*Calomys*): preliminary assessment of species, population and strain divergence

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Abstract

Investigated the morphometric variation of three species of the South American genus *Calomys* (*C. callidus*, *C. laucha*, *C. musculinus*). Canonical Variate Analysis (CVA), performed on 13 measurements from the left mandible, discriminates categorically the three species ($p < 0.01$) and allows for a posteriori identification of *C. laucha* and *C. musculinus*. These two morphologically very similar species are the vectors of the viral Argentine Haemorrhagic Fever. CVA performed on 4 natural populations and 2 laboratory strains from *C. musculinus* distinguishes successfully the groups ($p < 0.001$) showing a pattern of phenetic relationships congruent with the origins of the strains. These results suggest that multivariate morphometrics is a powerful tool to investigate on natural populations which are genetically determined to become viral infected.

Introduction

Systematics of the South American genus *Calomys* is still not completely clear. Some authors recognize different species in the genus (THOMAS 1916; TATE 1932; ELLERMAN 1941; CABRERA 1961; HERSHKOVITZ 1962; HONACKI et al. 1982). Out of the numerous putative species described, cytogenetic survey has been carried out and banding patterns have been established for 3 species only, namely *C. musculinus* (FORCONE et al. 1980), *C. laucha* (VITULLO et al. 1983), and *C. callidus* (VITULLO et al. 1984). *C. musculinus* shows a $2n = 38$ karyotype, whereas *C. laucha* has $2n = 64$ and *C. callidus* $2n = 48$. *C. musculinus* and *C. laucha* are morphologically very similar and live in sympatry in Buenos Aires province and in the South of Cordoba province (Fig. 1). MASSOIA et al. (1968) found some differences in the dental features and in tail length between *C. musculinus* and *C. laucha* which however are not useful to distinguish juvenile and/or oldest stages of the two species. Distinctness in karyological and allozyme patterns (GARDENAL et al. 1977) suggests that the two species are reproductively isolated and no hybridization occurs. *C. callidus* belongs to a group of forms which are morphologically larger (CABRERA 1961) and lives in the Mesopotamia and in the South of Santa Fe province where it overlaps the distribution area of the former two (Fig. 1).

These three species are the best known within the genus for their enormous importance as vectors of the *Junin* virus, the ethiological agent of Argentine Hemorrhagic Fever (PARODI et al. 1959; SABATTINI et al. 1977; SABATTINI and CONTIGIANI 1982). *C. laucha* and *C. musculinus* have been identified as the real vectors of this arenavirus, and they present viral persistence (SABATTINI et al. 1977). In overlapping areas *C. callidus* can be infected by these species and becomes a vector itself of the disease (VIDELA et al. 1985).

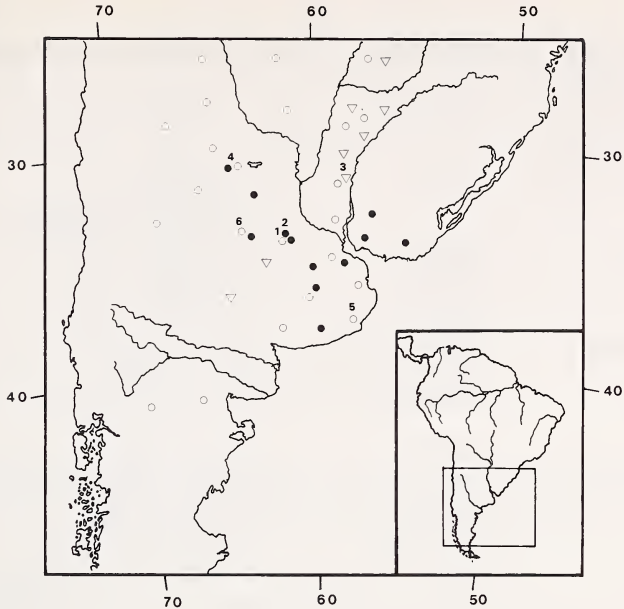


Fig. 1. Distribution area of the three species of *Calomys* based on data from literature and observations by the authors. *C. callidus* ▼; *C. laucha* ●; *C. musculinus* ○. Numbers indicate capture localities as follows: 1 = Pergamino Circulo 3; 2 = Pergamino Circulo 4; 3 = El Palmar; 4 = Laguna Larga; 5 = Chapadmalal; 6 = Laboulaye

In the present paper we use a multivariate morphometric approach to assess phenetic relationships among these three species in order to determine the best suitable functions for an a-posteriori classification of virus infected specimens. Moreover, we want to test whether multivariate morphometrics could detect any intra-specific differentiation among populations which could be consistent with their different sensibilities to become persistently infected by the virus. Multivariate morphometrics are an appropriate tool to investigate geographical variation and racial affinities (GOULD and JOHNSTON 1972; REYMENT et al. 1984; THORPE 1976), to discriminate closely related species (CORTI et al. 1985) and to identify house mouse laboratory strains (FESTING 1972, 1976; LEAMY 1975, 1977) and patterns of growth (ATCHLEY et al. 1985). Most of these studies use the mandible as source of data because it represents a pool of highly heritable traits (FESTING 1972, 1976; LEAMY 1975, 1977; THORPE et al. 1982; CORTI et al. 1985; HAUSSER 1985), is usually preserved in museum collections and for the accuracy and rapidity in recording procedures.

Material and methods

The analysis was performed on 100 specimens belonging to the three species, i.e. one population from *C. callidus*, 2 from *C. laucha*, 4 natural populations and two laboratory strains from *C. musculinus* (Tab.; Fig. 1). The specimens were sampled during ecological and virological surveys carried out through capture and recapture in epidemiological and non epidemiological areas. Only males were collected from the field not to alter the number of females in reproductive stage. Only one sex was therefore analysed in the present report. This does not particularly perturb the assessment of divergence and relative similarities between the groups since the species exhibit sexual dimorphism and separate analyses should have been performed for the two sexes.

Only adults were used to minimize the effect of allometric variation associated with growth.

13 measurements on the mandible of 100 specimens were recorded (Tab.), following the rapid and

Table

Species, populations and geographic location of the *Calomys* samples examined

Species	Population	Geographic location	Number of Specimens
<i>Calomys callidus</i>	El Palmar	32>.30' S, 58> W Prov. Entre Rios. Parque Nacional El Palmar	11
<i>Calomys laucha</i>	Pergamino Circulo 3	33>.50' S, 60>.32' W Prov. of Buenos Aires	11
	Pergamino Circulo 4	33>.50' S, 60>.32 W Prov. of Buenos Aires	10
<i>Calomys musculus</i>	Laguna Larga Cuartel 6	30>.25' S, 64>.25' W Prov. of Cordoba	9
	Chapadmalal	38>.10' S, 57>.38' W Prov. of Buenos Aires	9
	Laboulaye	34>.5' S, 63>.24' W Prov. of Cordoba	12
	Pergamino Circulo 3	33>.50' S, 60>.32 W Prov. of Buenos Aires	11
	vi/H CNEA strain	Commision Nacional de Energia Atomica. Buenos Aires	15
	vi strain	Istituto de Virologia Cordoba	12

accurate method described by FESTING (1972). To avoid lateral asymmetry only left mandibles were used. All data were converted to logarithms to render the relationship between them linear.

To assess distinction and phenetic relationships between species/populations Canonical Variate Analysis (CVA) was performed. CVA ordinales groups so that the between-group variation is maximized relatively to within-groups variation. Two different CVAs were performed, the first on the 7 natural populations of the three species, the latter on the 4 *C. musculus* populations plus the two laboratory strains.

Mahalanobis distances between group centroids were used to compute UPGMA phenograms (SNEATH and SOKAL 1973).

CVA was performed through the 7M program of the BMDP (1983) package. All data are available upon request from the authors.

Results

On the basis of CVA performed on the 7 natural populations the three species can clearly be distinguished. The separation of species and populations on the group means is given in Figure 2. All the between-species F-values are highly significant ($P < 0.01$). The classification matrix shows an average percent of correct classification of 95.97 % (*C. callidus* = 100 %; *C. laucha* = 95.24 %; *C. musculus* = 92.68 %). The first canonical axis separates the species in two distinct groups, one with the big *C. callidus* and one with the smaller *C. musculus* and *C. laucha*, according to classical taxonomy. The second canonical axis clearly discriminates *C. musculus* from *C. laucha* and no intermediate forms between them are observed.

CVA performed on the four natural populations and the two laboratory strains of *C. musculus* discriminates categorically ($p < 0.001$) the six groups. The first, second and third canonical axes absorb 49.27 %, 20.43 %, 12.24 % of the total variation respectively, to explain cumulatively the 81.24 % of the total dispersion (Fig. 3). The classification matrix shows a percentage of correct classification of 83.6 % (min. 66.2 %, max. 100 %).

The UPGMA computed on Mahalanobis distances between group means shows the pattern of phenetic similarity between populations and laboratory strains (Fig. 4).

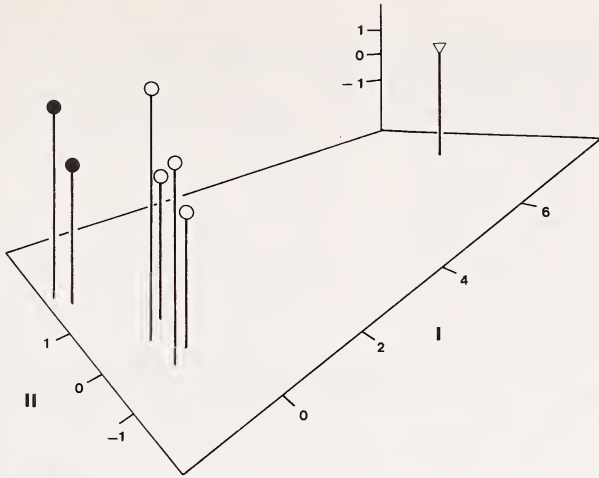


Fig. 2. Canonical Variate Analysis of the three species of *Calomys*. In this 3 dimensional plot the vertical axis is the third and final canonical variate. First axis evidences differences in size between *C. callidus* (▼) and *C. musculus* (○) and *C. laucha* (●)

Discussion

It is generally assumed that the species of the genus *Calomys*, on the basis of their size, are to be classified in two distinct groups, one with the big forms, including *C. callidus*, *C. callosus*, *C. venustus* (VITULLO et al. 1983, 1984; REIG 1984; ROLDAN et al. 1985), and one with the smaller ones, including *C. musculus*, *C. laucha*, *C. hummelineki* (THOMAS 1916; HUSSON 1960; HERSHKOVITZ 1962; MASSOIA et al. 1968). The phenetic distinction of *C. callidus* from *C. laucha* and *C. musculus* along the first canonical variate seems to fit well with the foregoing statements (Fig. 2). Second canonical variate describes more evidently changes between *C. musculus* and *C. laucha* which do not seem related with size (Fig. 2). Categorical morphometric distinction between these two full species is completely congruent with karyological (FORCONE et al. 1980; VITULLO et al. 1983, 1984) and alloenzymatic evidences (GARDENAL et al. 1977). This conclusion is supported by lack of intermediate forms due to hybridization in sympatric conditions as in Pergamino (Tab., Fig. 1).

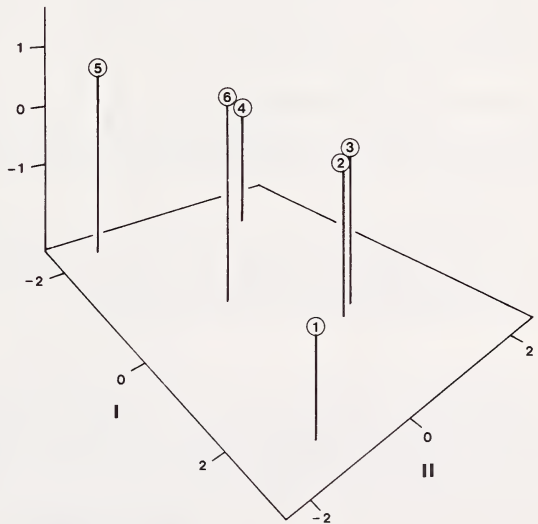


Fig. 3. Canonical Variate Analysis of the four natural populations and the two laboratory strains of *C. musculus*. The vertical axis is the third Canonical variate. Note the distinctness of the Pergamino (1) population in respect to the close relationship of Chapadmalal (2) and Laboulaye (3). vi (4) and vi/H (5) strains are more related to their common ancestor Laguna Larga (6)

Whilst karyology, alloenzymes and multivariate morphometrics contribute independently to fully identify the species, they do not allow to infer any phylogeny for the group. Further studies are necessary to elucidate whether the distinction in big and small *Calomys* species is a morphological convergence or a cladistic divergence.

Figures 3 and 4 summarize the results of the analyses performed on the four populations and the two laboratory strains from *C. musculus*. The isolate position of the Pergamino population (Fig. 3) and its high distance from the other groups (Fig. 4) is evident. Pergamino is the center of the endemic area of the Argentine Haemorrhagic Fever and probably this population responds differently to the virus in the wild.

Relative distinction and close clustering of the Laguna Larga population with the two laboratory strains (Fig. 3, Fig. 4) show a pattern of phenetic similarity which is congruent with the well known origin of these strains. "vi" strain (Tab.) originated from wild animals from Laguna Larga some 20 years ago and has been maintained in laboratory conditions for over 50 generations (DE VILLAFÑE 1981). "vi/H" strain (Tab.) derived from F 40 vi strain and was bred at the same room temperature and light system, but it is evident that differences in husbandry and mating systems were sufficient to establish a subline divergence within few generations. Strikingly, the morphometric divergence between these sublines is even stronger than the one observed in the two natural populations from Chapadmalal and Laboulaye (Fig. 3, Fig. 4) which are geographically far apart (Fig. 1).

The close morphometric similarity between Laboulaye (periendemic to the virus-infected area) and Chapadmalal (a non virus-endemic area) populations, might suggest that the latter could become persistently infected by the virus in the future. As a matter of fact, the endemic area progressively expanded northward in the past decade (WEISSENBACHER and DAMONTE 1983) and a southward expansion can be expected unless some mechanism would oppose it.

Our results suggest that mandible multivariate morphometrics represent a powerful tool to investigate on the dynamics of virus expansion, for species identification and for assessing population and subline divergences.

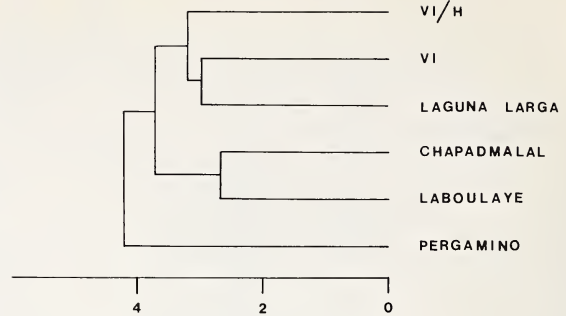


Fig. 4. This UPGMA clustering has been computed on Mahalanobis distances between group means and shows the phenetic relationships between the four natural populations and the two laboratory strains of *C. musculus*

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Zusammenfassung

Multivariate Morphometrie an Vespermäusen (Calomys): vorläufige Abschätzung von Arten, Populationen und Zuchtstammunterschieden

Die Variation von 3 Arten des südamerikanischen Genus *Calomys* (*C. callidus*, *C. laucha*, *C. musculus*) wurde morphometrisch untersucht. Dazu dienten 13 Maße an linken Mandibeln. Mit diesen konnten kanonische Analysen durchgeführt werden. Deutlich waren die Angehörigen der 3 Arten voneinander zu trennen, vor allem wurde eine Identifikation von *C. laucha* und *C. musculus*

möglich. Diese beiden morphologisch sehr ähnlichen Arten sind Träger des virusbedingten argentinischen Blutfiebers. Zusätzlich konnten durch kanonische Analysen 4 natürliche Populationen und 2 Laborzuchtstämme der Art *C. musculinus* deutlich unterschieden werden und eine Nähe der Zuchtstämme zu den Populationen ihrer Abstammung. Diese Ergebnisse deuten an, daß multivariate Morphometrie ein wichtiges Hilfsmittel darstellt, um natürliche Populationen zu untersuchen, die genetisch determiniert sind, durch Viren infiziert zu werden.

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Zur innerartlichen Proteinvariation bei der Waldmaus (*Apodemus sylvaticus*)

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Abstract

On the intraspecific variation of some proteins in the wood mouse (Apodemus sylvaticus)

Compared the alleles of the three polymorphic protein loci for transferrine, postalbumine, and glucose-6-phosphate dehydrogenase in more than 200 wood mice from different populations distributed from Sweden to Northwest Africa. Most of these alleles varied irregularly without any clear geographic pattern. An exception are the transferrines a and c. Allele c occurs in specimens of eastern Austria, northern Yugoslavia, Italy and Sardinia while allele a is found in individuals of Austria and the rest of the populations not yet mentioned.

The allelic patterns add arguments for the possible source of some isolated populations. For example the wood mice from Tunisia probably derived from Southwest Europe, not from Italy or the Eastern Mediterranean.

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